

EFFECTS OF PHYTOCHEMICAL DIVERSITY
ON THE HEALTH OF GROWING GOATS

A Thesis

Presented to the

Faculty of the College of Graduate Studies and Research

Angelo State University

In Partial Fulfillment of the
Requirements for the Degree
MASTER OF SCIENCE

by

REBECCA DEANN BURSON

May 2021

Major: Animal Science

EFFECTS OF PHYTOCHEMICAL DIVERSITY
ON THE HEALTH OF GROWING GOATS

by

REBECCA DEANN BURSON

APPROVED:

Dr. Cody B. Scott

Mr. Corey Owens

Dr. Chase Runyan

Dr. Andrew Siefker

May 2021

APPROVED:

Dr. Micheal W. Salisbury
Dean, College of Graduate Studies and Research

ACKNOWLEDGEMENTS

I am profoundly grateful for the decision I made to attend Angelo State University. I am fortunate to have received an education extending beyond the scope of a traditional classroom. My professors and colleagues have challenged me to broaden my way of thinking and to take advantage of opportunities that have allowed me to glean new skills and insights that will translate into future career paths; for that, I am forever indebted.

Above all, I recognize I would not be where I am today without the salvation of the Lord. I am blessed with strengths and talents that exceed my own abilities and have been afforded to me only by the grace of God. The faith I have in a compassionate God that has led me where I am today would not be possible without the guidance of a family that has provided me with a lifetime of love and encouragement. I am beyond thankful for their endless support through all chapters of my life.

I would also like to extend thanks to the many individuals assisting with my research at the Management, Instruction, and Research Center. My project would not have been possible without the flexibility, facilities, and expertise provided by the ASU ranch manager, Cody Riddle. Thank you to Dr. Cody Scott, Clint Smith, and Ryan Kennedy for helping me harvest redberry juniper and shin oak leaves. I would also like to express my thanks to Madonna Bullard, Kalyn Stephens, and Ashley McGinnis for assisting with the feeding process. While I am very appreciative for all help offered by each of the undergraduate and graduate students at the MIR Center, I am even more blessed by their friendships, which have enriched my experience at this university.

Finally, I would be remiss not to express my gratitude for the opportunity to study under a respected leader in the Range Science and Management industry, Dr. Cody Scott. Your mentorship has been invaluable. I believe that my education was enhanced considerably by your knowledge and perspective. Further, I must acknowledge many thanks to Corey Owens for providing much assistance in all aspects of data collection, as well as giving me opportunities to grow in the classroom by allowing me to teach his Range Improvements lab on multiple occasions. Thank you to Dr. Chris Womack and Dr. Andrew Siefker for serving on my committee and Dr. Chase Runyan for stepping in to assist with my thesis defense.

ABSTRACT

Forage diversity in the grazing diet of ruminants is an area of growing interest as it could provide key nutritional benefits in animal production. The objective of this study was to determine if there is a positive correlation between the presence of a wide range of chemically rich forages and the health of growing goats. This idea was assessed by comparing physiological responses to vaccination challenges of animals fed differing diets. In two separate trials, goats were placed in individual pens and fed one of three treatments in a completely randomized design. Treatments in Trial 1 consisted of redberry juniper and shin oak. In Trial 2, goats were fed totally mixed rations containing grape and blueberry pomace at an inclusion rate of 20%. Average daily gain, intake, and blood chemistries were assessed following a vaccination health challenge. In Trial 1, goats fed shinoak had higher ($P<0.05$) globulins and total protein of blood serum. Trial 2 revealed no differences in ADG, intake, or blood parameters evaluated. In conclusion, phytochemical compounds may have the ability to enhance the health of goats, but the health effects may be dependent upon the source and structure of the chemical components offered.

TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENTS	iii
ABSTRACT	v
TABLE OF CONTENTS	vi
LIST OF FIGURES	viii
LIST OF TABLES	ix
INTRODUCTION	1
OBJECTIVES	3
LITERATURE REVIEW	4
<i>Plant Secondary Metabolites</i>	4
<i>Tannins</i>	5
<i>Juniper as a Ruminant Feedstuff</i>	5
<i>Mechanisms of Diet Selection</i>	7
<i>Management Based Solutions</i>	7
METHODS	9
Trial 1	9
Trial 2	12
RESULTS	17
Trial 1	17
Trial 2	22
DISCUSSION	27
Trial 1	27

Trial 2.....	28
IMPLICATIONS	31
LITERATURE CITED	32
APPENDIX.....	36
VITA.....	37

LIST OF FIGURES

	Page
Figure 1. Average Daily Gain (ADG) in kg of wether goats in pounds across shin oak, redberry juniper, and control treatment groups in Trial 1.....	18
Figure 2. Average Daily Intake ($\text{g} \cdot \text{kg}^{-1}$ BW) of juniper and shin oak plant treatments by goats in Trial 1. Goats in both treatment groups were allowed 7 days prior to the start of the trial to accept plant treatments as a dietary feedstuff. Animals were allowed 30 minutes to consume treatment leaves prior to receiving the basal ration.	19
Figure 3. Globulin levels ($\text{g} \cdot \text{dL}^{-1}$) detected in serum of goats 0,7,14, and 21 days post-feeding.....	20
Figure 4. Average total protein ($\text{g} \cdot \text{dL}^{-1}$) found in blood serum of wether goats across shin oak, control, and redberry juniper treatment groups in Trial 1. Results were averaged across the 14-day duration of the study..	21
Figure 5. Average Daily Gain (ADG) in kg of wether goats in pounds across control, blueberry, and grape treatment groups in Trial 2.....	23
Figure 6. Average daily intake ($\text{g} \cdot \text{kg}^{-1}$) by individually stalled goats of control, blueberry, and grape totally mixed rations fed at 3% BW for 21 days.	24
Figure 7. Globulin levels ($\text{g} \cdot \text{dL}^{-1}$) detected in serum of goats on Days 0,7, and 14 of Trial 2. Treatment rations were fed throughout the span of this trial.....	25
Figure 8. Total protein ($\text{g} \cdot \text{dL}^{-1}$) found in blood serum of wether goats across control, blueberry, and grape treatment groups in Trial 2. Results were averaged across the 14-day duration of the study.	26

LIST OF TABLES

	Page
Table 1. Ingredients and nutrient content of the basal ration fed to goats to meet maintenance requirements.....	10
Table 2. Ingredients and nutrient content of the grape pomace ration fed to goats to meet maintenance requirements	14
Table 3. Ingredients and nutrient content of the blueberry pomace ration fed to goats to meet maintenance requirements	15
Table 4. Ingredients and nutrient content of the control ration fed to goats to meet maintenance requirements.	16

INTRODUCTION

Even 200 years ago English poet William Cowper knew that “Variety is the very spice of life, that gives it all its flavor.” While there are a multitude of facets in which this statement rings true, the importance of a diversity of forages in the grazing diet of ruminants is an area of growing interest as it could provide key nutritional benefits in animal production. The concern over antibiotic and anthelmintic resistance in livestock production has become a prevalent issue facing the agricultural industry. The emergence of natural solutions could prove to be a viable alternative to modern medicine, potentially reducing costly impacts of epidemics to both the animal and the producer.

In the Edwards Plateau and western regions of Texas, phytochemical bearing compounds such as terpenes present in juniper (*Juniperus* sp.), tannins in oaks (*Quercus* sp.) and a diversity of compounds in honey mesquite (*Prosopis glandulosa* Torr.) are readily available to the ruminant forager. Provenza et al. (2015) have alluded that the presence of phytochemicals, like terpenes and tannins, in a nutritionally diverse landscape may attune the appetite of foraging species to consume plants that will not only nourish but possibly promote well-being and prevent disease. Unfortunately, the negative digestive consequences of the consumption of plant secondary metabolites at highly concentrated doses has obscured their medicinal value to herbivores. Additionally, phytochemical compounds may be unpalatable to prospective herbivores and quickly induce satiety (Provenza and Villalba 2010). A rangeland environment with the presence of a variety of bioactive forages presents

a unique conundrum to the producer; they must develop grazing systems that can allow animals to learn and capitalize on the beneficial properties of secondary components without adversely impacting production (Provenza and Villalba 2010).

OBJECTIVES

The goal of this study was to determine if there is a positive correlation between the presence of a wide range of chemically rich forages and the health of growing goats. This idea was assessed through the comparison of physiological responses to vaccination challenges of animals fed differing diets.

LITERATURE REVIEW

Plant Secondary Metabolites

The thousands of chemical components found in plant structures can be simplified into two categories of compounds: primary and secondary (Provenza et al. 2015). Under adequate rangeland conditions, plant forages contain enough primary compounds (carbohydrates, amino acids, lipids, vitamins and minerals) to sustain grazing animal diets. Additionally, these primary chemical compounds allow for photosynthesis and promote the development of the plant. A category of “secondary” components exists in plant metabolism and is unrelated to basic functional development. Researchers such as Rosenthal and Berenbaum (1992) have studied the roles of these secondary metabolites, alternately referred to as “phytochemicals,” and have found that they are more than mere byproducts of plant metabolism but serve functions such as recovery, attraction of pollinators, and protection from consumption by herbivores. Due to their role as a defense mechanism against herbivores, phytochemicals may illicit a negative flavor-feedback response when selected. Likewise, these same plant secondary compounds that injure mammalian predators at highly concentrated levels can adversely affect parasites, bacteria and fungi living within a host when eaten in lesser quantities (Lozano 1998).

Over 100,000 complex combinations of naturally occurring secondary metabolites can be derived from isoprenoid, phenylpropanoid, alkaloid or fatty acid/polyketide pathways (Dixon 2001). Kabera (2014) recognized that these plant secondary metabolites could be broken down into three predominant classes: alkaloids, phenolics and terpenoids. Classified according to biosynthetic properties, each of these categories contain some bioactive

compounds with potentially healing properties, making them key ingredients in essential oils to pharmaceuticals (Costa et al. 2012).

Tannins

Tannins are a phenolic compound widely available to ruminants in many countries. Tannins may be classified into two categories: hydrolyzable (HT) or condensed (CT). The presence of CT is more frequently found than HT in shrubs, trees, and legumes (Min et al. 2003); therefore, this proposed study will focus solely on tannins in their condensed form.

The molecular weight and structure of both condensed tannins (CT) and proteins influences hydrophobic and hydrogen bonding of proteins with tannins in a manner that can reverse pH, decreasing forage degradation in the rumen without negatively impacting synthesis of microbial protein. The molecular structures of condensed tannins vary, as does their rate of consumption by ruminants and their effects on animal health (Min et al. 2003). Notably, potential benefits of CT to the animal include reduction of internal parasite load (Min and Hart 2003) bloat alleviation (McMahon et al. 2000), and increased protein efficiency (Waghorn et al. 1998).

Juniper as a Ruminant Feedstuff

The invasive species redberry (*Juniperus pinchotii* Sudw.) and ashe (*Juniperus asheii* Buch.) juniper (also referred to as blueberry juniper) are no stranger to the Edwards Plateau region of Texas. Unlike most herbaceous plants, the moderate levels of crude protein and energy of blueberry juniper remain relatively constant year-round. Ideally, this would make the woody species a practical nutrient source for grazing animals in the fall and winter months. However, animals are reluctant to select for juniper. Terpene-containing essential

oils commonly found in the needles, branches and berries of the native trees are likely the cause of herbivores' aversion to juniper (Launchbaugh et al. 1997).

Through the metabolic and digestive mechanisms of the ruminant system, liberated essential oil compounds from juniper will either be subject to microbial detoxification in the rumen or be absorbed through the walls of the rumen and small intestine in transportation to the liver. Once in the liver, terpenes undergo biotransformation to make plant lipophilic substances more water soluble en route to urinary or fecal excretion (Smith 1992). Past studies of invasive plant inclusion in the diet have shown that intake of juniper may be increased through preconditioning strategies (Dunson et al. 2007) and supplementation of protein (George et al. 2010).

To test the correlation between goats eating more juniper when provided supplemental protein, George et al. (2010) used weaned Boer-cross goats in an experiment with 5 treatments: 1) cottonseed meal (CSM), 2) cottonseed meal and dried distiller's grains (CSMD), 3) soybean meal (SBM), 4) soybean meal and dried distiller's grains (SBMD), and 5) a control group fed alfalfa pellets with no additional protein source. Redberry juniper leaves were offered following fed protein supplements. Results from this study concluded that goats receiving additional protein increased their intake of juniper compared to goats only fed alfalfa pellets. Implications of this study are relative to producers in juniper-containing rangelands because all viable nutrient options must be fully exploited during winter months and times of drought to remain profitable.

Mechanisms of Diet Selection

Feeding behaviors of herbivores depend upon the feeding system and environment. Contrary to monoculture diets and confinement, selective and purposeful habits may be developed when animals are offered an abundance of plant species for consumption (Provenza et al. 2015). When a variety of feedstuffs exist, the relationships between mammals and the forages they consume are anything but random. Positive or negative digestive feedback from interactions between the grazing animal and the forages they eat facilitate this decision-making process. Herbivores can choose to avoid certain classifications of forages that are toxic, as well as knowingly consume some levels of toxin-containing plants (Freeland and Janzen 1974). Lisonbee et al. (2009) illustrated this theory in parasitized lambs, finding an increased intake of tannin containing supplement by lambs when a high parasite load was present compared to lambs with a low parasitic burden. A sophisticated rumen system containing microbial enzymes that allow for gut detoxification may allow for adaptation to potentially detrimental chemical compounds (Freeland and Janzen 1974). Through learned behaviors gained from these nutritional feedback experiences in a biodiverse landscape, animals may be able to select for vegetation containing secondary compounds when needed to self-medicate (Villalba and Provenza 2009).

Management Based Solutions

If naturally occurring plant secondary metabolites have both negative and positive impacts to ruminants, can grazing systems capitalize on the good without the detrimental consequences of the bad? A simple answer to the problem may be found in plant diversity. The inherent variances of chemical structures in different compounds and their pathways in

the body allow them to be less harmful when multiple compounds are selected together, as opposed to larger quantities of a singular toxic species (Villalba and Provenza 2009). A study of sheep conducted by Villalba et al. (2004) showed that lambs conditioned to eat tannin, terpene, or oxalate containing feedstuffs consumed more if they could choose multiple foods simultaneously. In contrast, intake decreased when only one source of plant secondary metabolites was offered. Additionally, lambs provided three options of toxins consumed more than those given two choices of toxins. The ability of animals to learn from experiences and acknowledge the value of phytochemicals in a diverse landscape may be a way for management programs to benefit from their health promoting properties without sacrificing well-being.

METHODS

Prior to experimentation, all procedures were reviewed and approved by Angelo State University's Institutional Animal Care and Use Committee (Approval # 2020 – 105). This study was conducted at the Management, Instruction, and Research (MIR) Center in San Angelo, Texas (Lat: 31° 34' 8.99" N, Long: 100° 32' 26.399" W). The initial trial was performed in the Fall of 2020 to evaluate animal immune response to phytochemical-containing plant treatments. A follow-up trial was carried out in the Spring of 2021 to evaluate animal immune response specific to condensed tannins compounds offered in the form of grape and blueberry pomace.

Trial 1

Freshly weaned Spanish wether goats weighing approximately $25 \text{ kg} \pm 0.63$ and an estimated 150 days old were obtained from the Owens Ranch in Barnhart, Texas. Following an acclimation period of 14 days, weaned Spanish wether goats ($n=30$) were weighed and randomly allocated to individual treatment pens (1 m by 1.5 m) and equally assigned one of three diets to be applied for 28 days. The treatments were: i) Redberry juniper (*Juniperus pinchotii* Sudw.) ii) Shinnery Oak (*Quercus havardii* Rydb.) and iii) a control group fed only a basal diet (Table 1).

Wethers were allotted 7 days to acclimate to pens and basal rations before receiving treatments. Phytochemical containing treatment rations were completely novel to the animals at the time of initial exposure. Prior to experimentation, animals allocated to groups containing plant secondary compounds in Treatment 1 and Treatment 2 were offered their

Table 1. Ingredients and nutrient content of the basal ration fed to goats to meet maintenance requirements.

Ingredients/Nutrients	As fed (%)
Alfalfa Pellets	10.0
Cotton Seed Meal	12.5
Cottonseed hulls	31.5
Cane molasses	3.5
ASU premix	2.5
Corn	40.0
DE	2.6 Mcal/kg
TDN	59.0
Crude Protein	14.5
Crude Fiber	14.2

respective forages for 7 days to condition an acceptance as a dietary feedstuff. Following conditioning, a 14-day experimental feeding period commenced.

Daily feed intake was monitored throughout the project. Remaining orts from the previous feeding were weighed at approximately 0800 each day. Following collection of any refusals, diet treatments (50 g) were presented to animals allocated to Treatments 1 and 2 for 30 minutes. After 30 minutes, remaining shin oak and juniper orts were collected and weighed. All animals then received a basal diet ($750 \text{ g} \cdot \text{hd}^{-1} \cdot \text{day}^{-1}$). Fresh water was provided *ad libitum*. Average daily gain (ADG) was evaluated as a measure of animal performance. Fecal samples were collected to measure egg counts at the beginning and end of the experiment to analyze approximate internal parasite load.

The experiment expired on Day 14 and individual pen feeding of treatments and basal rations ceased. Immediately following the removal of goats from individual stalls, all animals were weighed and subjected to a health challenge using an Enterotoxemia Type C and D vaccination at the recommended dosing rate to evaluate each animal's immunological response. No kids used in this study were previously exposed to the vaccination. Whole blood and serum were collected and analyzed on days 0, 7, 14, and 21 post-vaccination. During this time, all animals continued to receive the basal ration at 3% BW.

Body temperature was recorded for all animals before administering vaccine and at 48- and 72-hours post dosing. Likewise, a visual assessment was performed at 0, 48, and 72 hours. All bloodwork collected as part of this experiment was shipped to and analyzed by the Texas A&M Veterinary Medical Diagnostic Laboratory in Canyon, Texas.

Data was analyzed using repeated measures analysis with treatment (juniper, oak, control) as the main effect. Animals nested within treatments served as replications and day of collection as the repeated measure. Means were separated using Tukey's Protected LSD when $P \leq 0.05$. Data was analyzed using JMP (SAS 2007).

Trial 2

Freshly weaned Spanish/Boer cross wether goats weighing approximately $25 \text{ kg} \pm .61 \text{ kg}$ and an estimated 150 days old were obtained from the Owens Ranch in Barnhart, Texas. Weaned wether goats ($n=30$) were weighed, randomly allocated to treatment pens, and equally assigned one of three diets to be applied for 28 days. The treatments offered were: i) Grape pomace based total mixed ration (TMR) (Table 2) ii) Blueberry pomace based TMR (Table 3) and iii) TMR with no tannin containing component (control) (Table 4). Individual animal rations were formulated at 3% BW to meet daily maintenance requirements (NRC 2007).

Animals were given 7 days to acclimate to pens and to condition an acceptance to a new dietary feedstuff. Following diet conditioning, a 21-day trial began. Daily feed intake was monitored throughout the project. Remaining orts from the previous feeding were weighed and assessed at approximately 0800 each day, prior to delivering fresh feed. Fresh water was provided *ad libitum*. Average daily gain (ADG) was evaluated as a measure of animal performance.

After the initial 7-day acclimation period, all animals were subjected to a health challenge using an Enterotoxemia Type C and D vaccination at the recommended dosing rate to evaluate each animal's immunological response. No kids used in this study were

previously exposed to the vaccination. Unlike Trial #1, treatment rations continued to be fed after administration of the vaccine and throughout weekly blood sampling process. Whole blood and serum were collected and analyzed on days 0, 7, 14, and 21 post-vaccination. All bloodwork collected as part of this experiment was shipped to and analyzed by the Texas A&M Veterinary Medical Diagnostic Laboratory in Canyon, Texas.

Due to a lack of significance in Trial #1, body temperatures and visual inspections were not recorded before or after vaccination was administered. All animals were dewormed prior to initiation of the study. No fecals were collected or analyzed as part of Trial #2.

Data was analyzed using repeated measures analysis with treatment (blueberry, grape, control) as the main effect. Animals nested within treatments served as replications and day of collection as the repeated measure. Means were separated using Tukey's Protected LSD when $P \leq 0.05$. Data was analyzed using JMP (SAS 2007).

Table 2. Ingredients and nutrient content of the grape pomace ration fed to goats to meet maintenance requirements.

Ingredients/Nutrients	As fed (%)
Alfalfa Pellets	10.0
Cotton Seed Meal	12.5
Cottonseed hulls	11.5
Grape Pomace	20.0
Cane molasses	3.5
ASU premix	2.5
Corn	40.0
DE	2.6 Mcal/kg
TDN	59.0
Crude Protein	14.5
Crude Fiber	14.2

Table 3. Ingredients and nutrient content of the blueberry pomace ration fed to goats to meet maintenance requirements.

Ingredients/Nutrients	As fed (%)
Alfalfa Pellets	10.0
Cotton Seed Meal	12.5
Cottonseed hulls	11.5
Blueberry Pomace	20.0
Cane molasses	3.5
ASU premix	2.5
Corn	40.0
DE	2.6 Mcal/kg
TDN	59.0
Crude Protein	14.5
Crude Fiber	14.2

Table 4. Ingredients and nutrient content of the control ration fed to goats to meet maintenance requirements.

Ingredients/Nutrients	As fed (%)
Alfalfa Pellets	10.0
Cotton Seed Meal	12.5
Cottonseed hulls	31.5
Cane molasses	3.5
ASU premix	2.5
Corn	40.0
DE	2.6 Mcal/kg
TDN	59.0
Crude Protein	14.5
Crude Fiber	14.2

RESULTS

Trial 1

After initial acclimation to the basal diet, all animals willingly consumed feed rations in entirety daily. Average daily gain (ADG) was similar for goats receiving the control ration ($0.16 \pm .04$ lbs.) compared to goats consuming treatments of shin oak or juniper ($.13 \pm .04$, $.12 \pm .04$ lbs.) (Fig. 1). Average daily treatment intake of shin oak was greater than the daily intake of juniper (Fig. 2).

Statistical differences in globulin levels ($\text{g} \cdot \text{dL}^{-1}$) were evident for animals consuming the shin oak ration on a treatment by time interaction ($P \leq .05$) (Fig. 3). Globulin levels for animals receiving the redberry juniper and control diets were similar. Serum data indicated that the treatment effect on total protein ($\text{g} \cdot \text{dL}^{-1}$) was statistically different for animals consuming shin oak compared to other treatment groups (Fig. 4). Although bloodwork sampling error prevented white blood cell (WBC) and red blood cell (RBC) data from being analyzed on day 0 and 7, treatment impact tended to effect WBC levels in goats ($P \leq .06$). Twenty-one days post-trial, control animals exhibited higher WBCs than animals in the shin oak and juniper treatment groups (data not shown). Statistical analysis was not evaluated on data collected for red blood cells (RBC).

No reportable significance was detected between treatments on body temperature before or after administration of Enterotoxemia Type C and D vaccination. During the trial, multiple animals experienced symptoms of coccidiosis and high internal parasite loads. All goats were dosed with a 5-day Corid regimen and administered 3 ml of Valbazen. Because these treatments would have skewed the data, subsequent fecal egg counts were not performed.

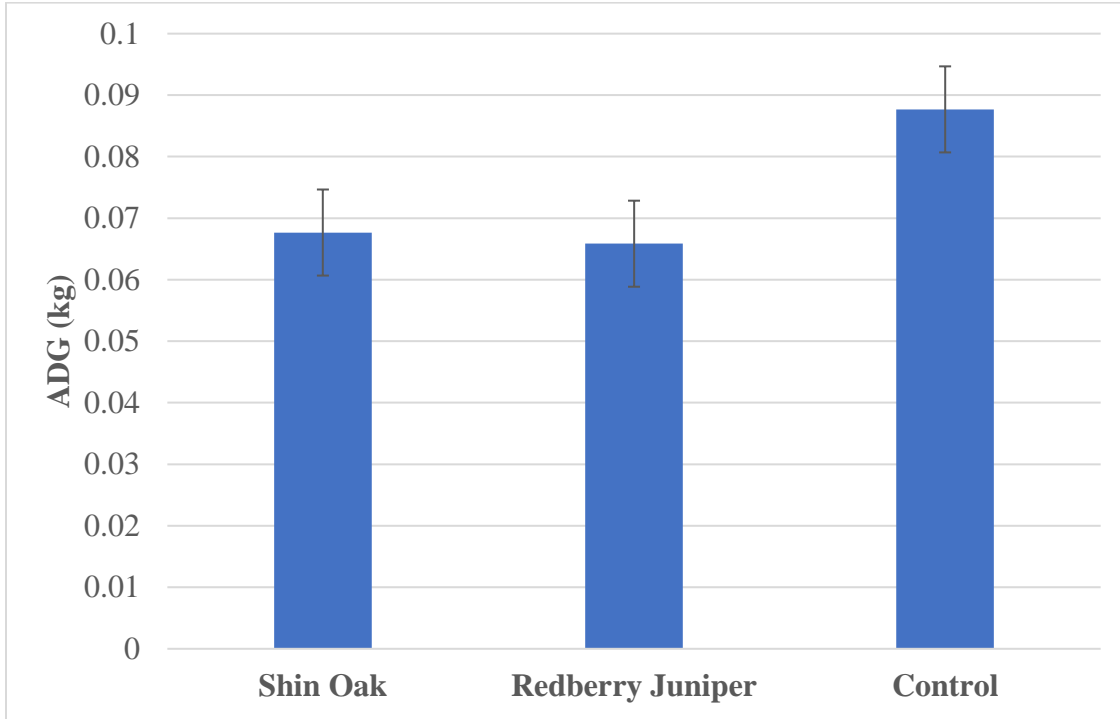


Figure 1. Average Daily Gain (ADG) of wether goats in kilograms across shin oak, redberry juniper, and control treatment groups in Trial 1.

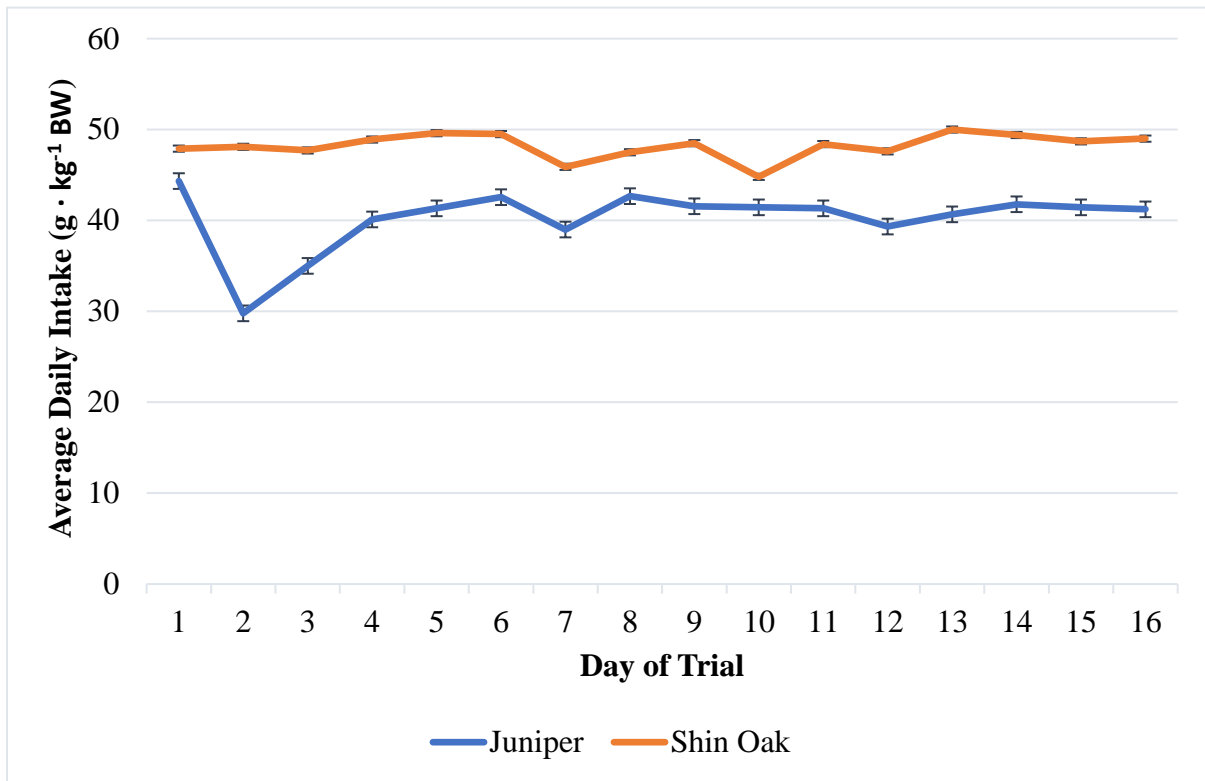
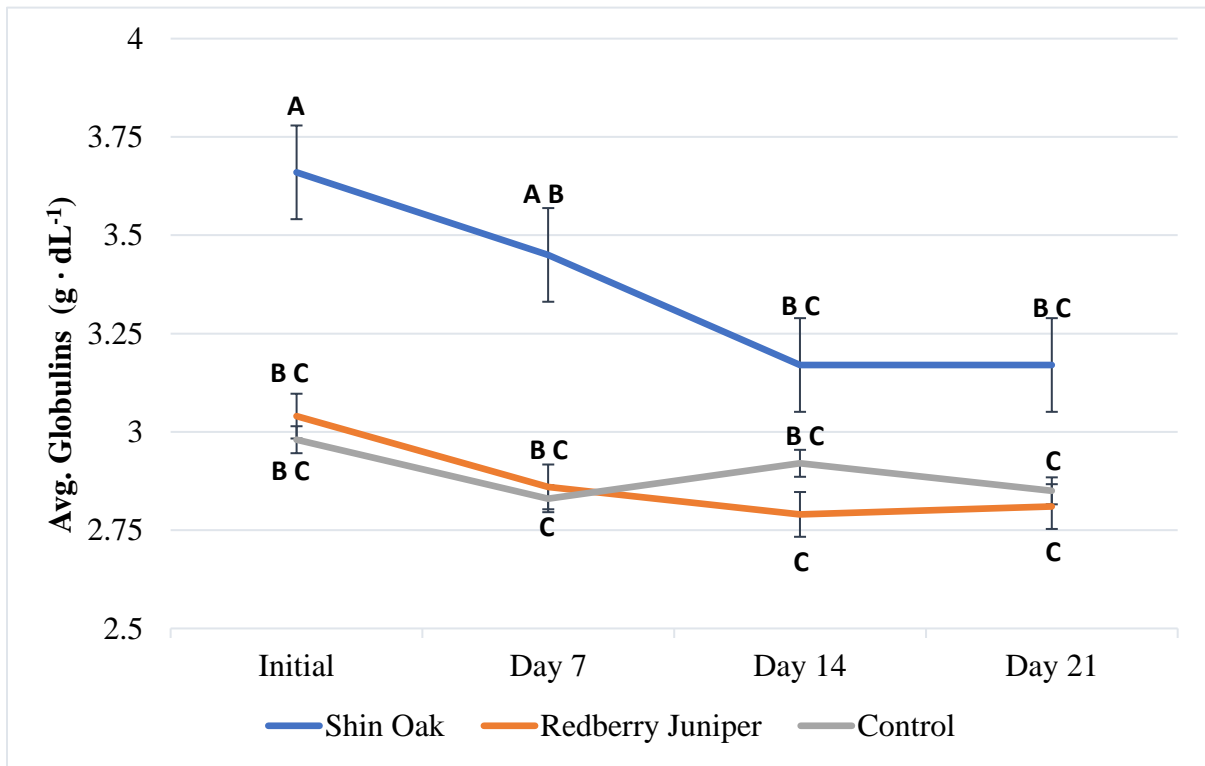
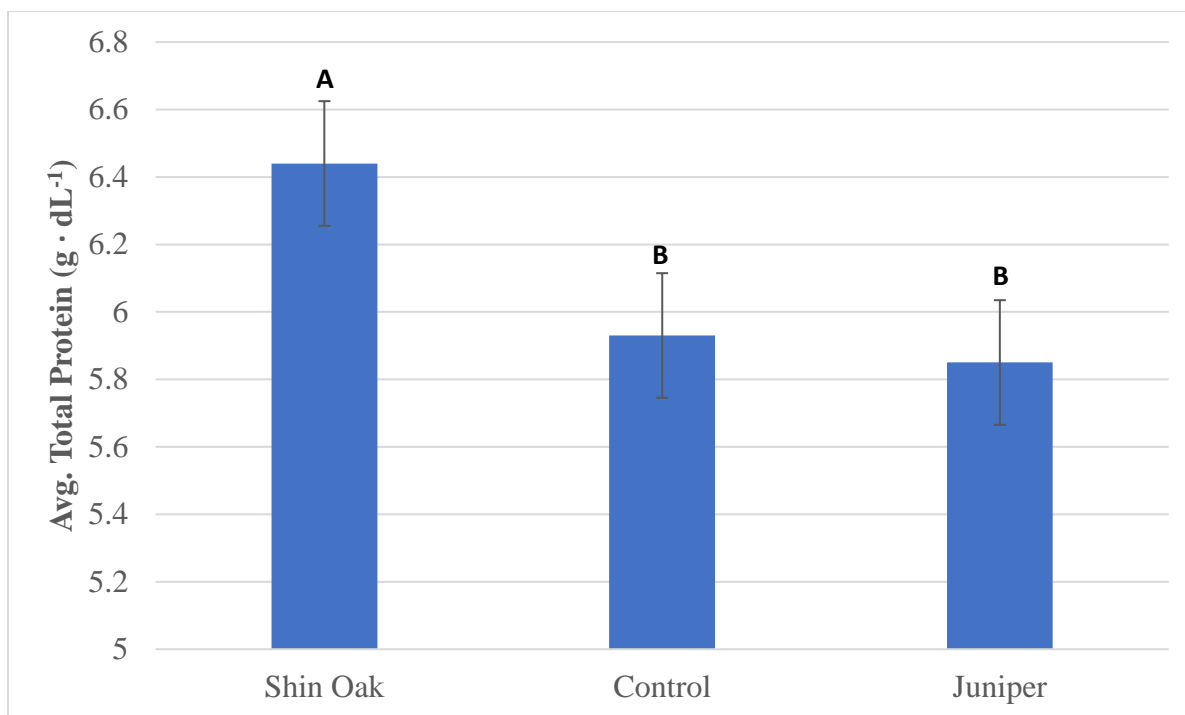


Figure 2. Average Daily Intake ($\text{g} \cdot \text{kg}^{-1} \text{BW}$) of juniper and shin oak plant treatments by goats in Trial 1. Goats in both treatment groups were allowed 7 days prior to the start of the trial to accept plant treatments as a dietary feedstuff. Animals were allowed 30 minutes to consume treatment leaves prior to receiving the basal ration.



^{A-C} Those columns sharing a letter of significance do not differ within day ($P < 0.05$).

Figure 3. Globulin levels ($\text{g} \cdot \text{dL}^{-1}$) detected in serum of goats 0,7,14, and 21 days post-feeding.



^{A-B} Those columns sharing a letter of significance do not differ ($P < 0.05$).

Figure 4. Average total protein ($\text{g} \cdot \text{dL}^{-1}$) found in blood serum of wether goats across shin oak, control, and redberry juniper treatment groups in Trial 1. Results were averaged across the 14-day duration of the study.

Trial 2

Average daily Gain and intake ($\text{g} \cdot \text{kg}^{-1} \text{ BW}$) was similar across all treatment groups (Figs. 5 and 6). Goats willingly consumed all three rations after the initial acclimation period. Unlike Trial 1, no statistical differences were detected from bloodwork collection data. Treatments in Trial 2 had no effect on globulin (Fig 7.), or total protein values (Fig. 8.) of serum. Whole blood data for total WBC and RBC values revealed comparable results with no differences detected across control, grape pomace, or blueberry pomace treatments.

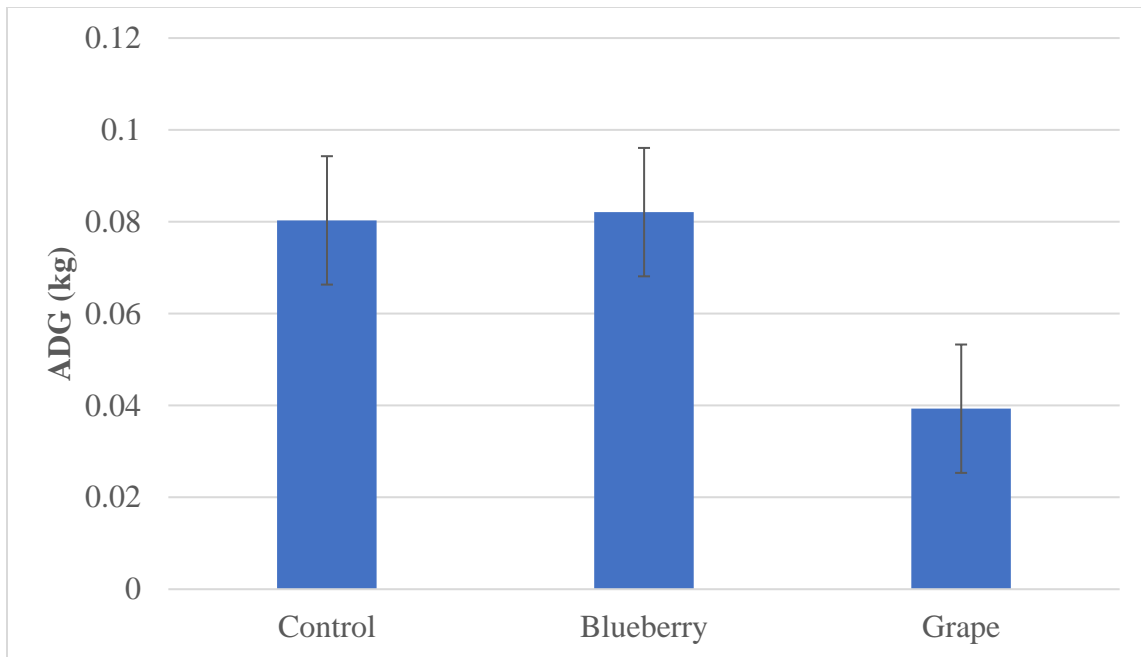


Figure 5. Average Daily Gain (ADG) of wether goats in kilograms across control, blueberry, and grape treatment groups in Trial 1.

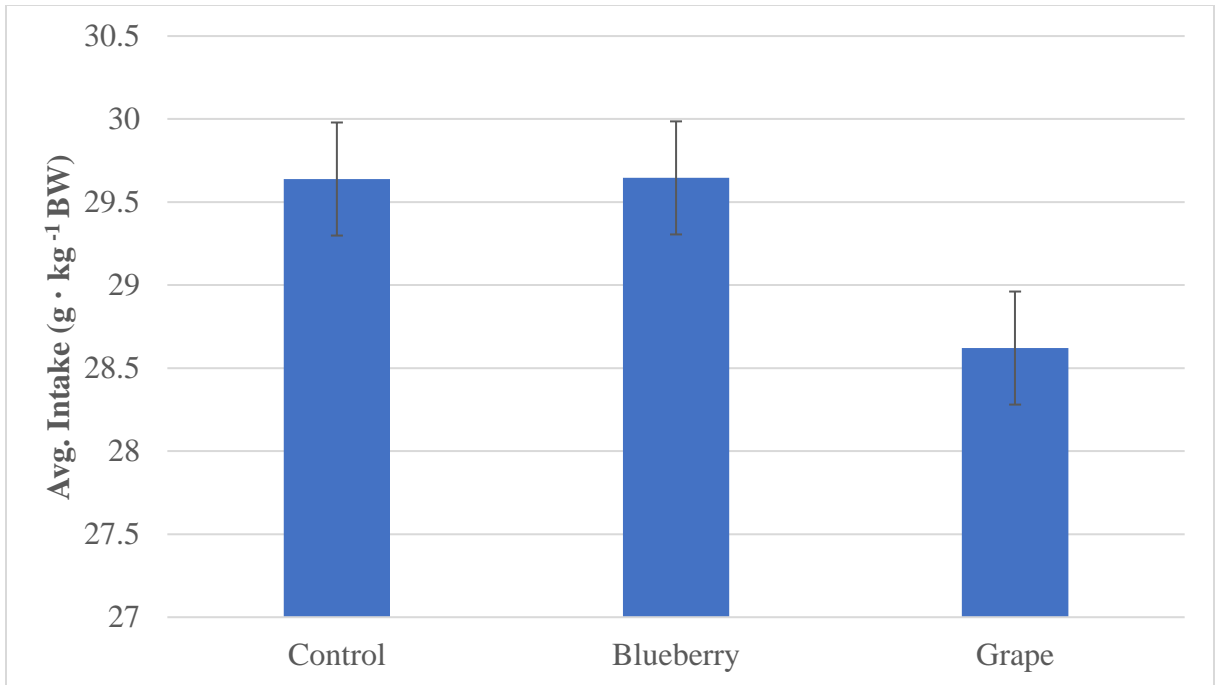


Figure 6. Average daily intake ($\text{g} \cdot \text{kg}^{-1}$) by individually stalled goats of control, blueberry, and grape totally mixed rations fed at 3% BW for 21 days.

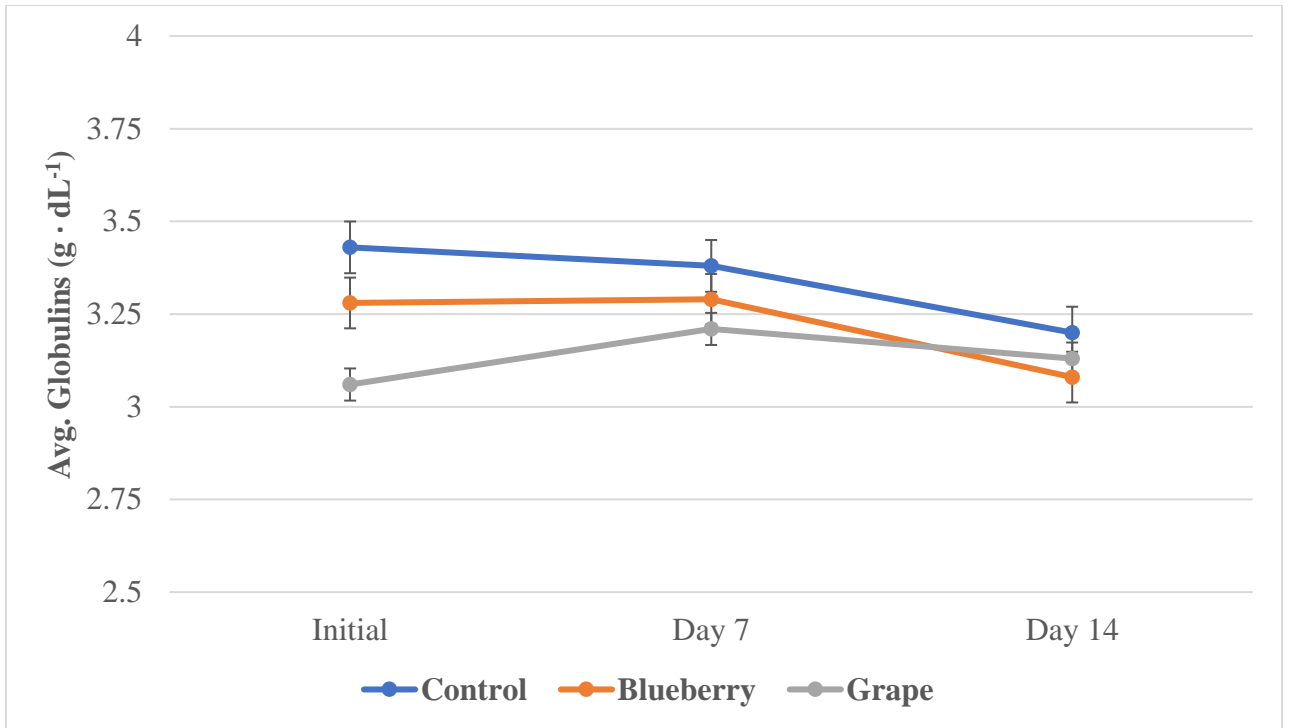


Figure 7. Globulin levels ($\text{g} \cdot \text{dL}^{-1}$) detected in serum of goats on Days 0,7, and 14 of Trial 2. Treatment rations were fed throughout the span of this trial.

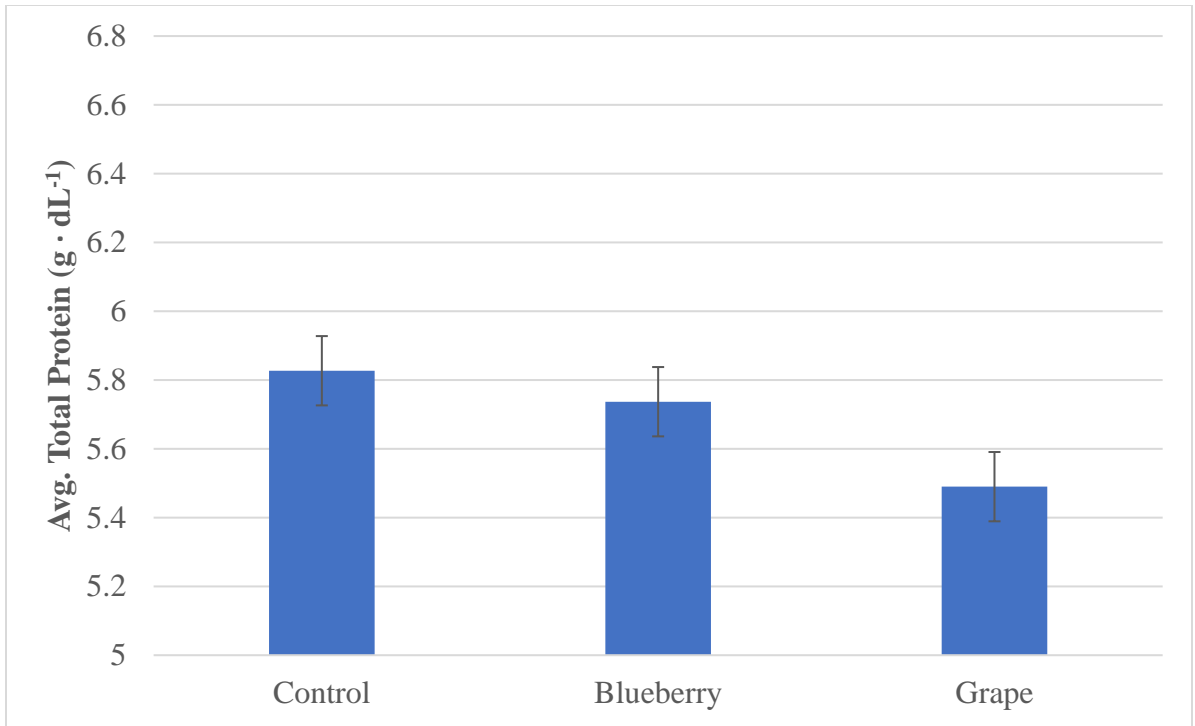


Figure 8. Total protein ($\text{g} \cdot \text{dL}^{-1}$) found in blood serum of wether goats across control, blueberry, and grape treatment groups in Trial 2. Results were averaged across the 14-day duration of the study.

DISCUSSION

Trial 1

In this study, treatment with secondary compounds had no effect on intake of basal rations or ADG. Most animals readily consumed feed daily and had comparable rates of gain at completion of the experiment. Intake of shin oak by goats increased more quickly than acclimation to juniper treatment. An enhanced rate of shin oak intake was maintained throughout the duration of the project (Fig. 2). Apparently, the phytochemical profile and terpene content of redberry juniper was more aversive to goats than the CT secondary compounds found in shin oak leaves. During the treatment acclimation period, the onset of coccidiosis in some animals was presented through symptoms including loss of appetite, lethargy, and diarrhea. Even when this presence of illness caused goats to avoid the basal rations, animals in the shin oak group still consumed leaves while animals in the redberry juniper treatment group did not.

Blood serum evaluations in this project indicated a statistically higher content of globulins and total protein in the shin oak treatment group at completion of the experiment. Perhaps the CTs found in shin oak leaves enhanced the response of the immune system to the challenge of the Enterotoxemia Type C and D vaccination. On Day 14 of post-trial sampling, globulin levels experienced a decline. On Day 0 of data collection, animals were removed from pens and feeding of treatments subsided. This drop would be explained by the lack of treatment effect 14 days post-consumption. Whole blood sampling errors occurred during the project making data unattainable on Days 0 and 7 post-experiment. Results achieved on Days

14 and 21 could show a trend towards higher total WBC counts of animals in the control and shin oak groups, but a statistical difference could not be confirmed due to the lack of data.

The onset of coccidiosis and internal parasites apparent by presence of bottle jaw in some animals required immediate treatment during the study. Goats were given a 5-day Corid regimen and dosed with 3 ml of Valbazen on Day 1 of the trial. Although fecal egg counts would have enriched the scope of this experiment, previous research indicates a correlation between increased CTs in the diet and a reduction in internal parasites (Min and Hart 2003).

Trial 2

Results obtained from Trial 1 of the project prompted a follow-up study to be carried out for evaluation of blood parameters specific to an increase of CT content in the diet. This idea was facilitated by the inclusion of grape and blueberry pomace at 20% of totally mixed treatment rations. The condensed tannin content of grape pomace is high, with blueberry pomace showing similar levels (Brenes et al. 2008, Ross et al. 2017).

Like Trial 1, results from this study indicated no adverse effects on weight gain of animals between treatments. ADG results were positive and similar between all treatment groups. Although there was a trend for goats to consume more control and blueberry pomace than the grape ration, results were not significant.

Blood parameters evaluated, however, did not obtain comparable findings to those in Trial 1. No statistical differences were seen in blood serum or whole blood data. While the actual cause for this inconsistency is unknown, potential explanations may be offered. A possible suggestion for the discrepancies found in between experiments could be explained

by the highly variable structure of phenolic compounds (Cheeke 1988). Phenolic compounds are classified as tannins, either condensed or hydrolysable based on their general chemical structure. Condensed tannins can produce a wide variety of responses, depending on the specific chemical structure. For example, some condensed tannins chelate with saponins thereby reducing the likelihood of bloat on wheat pastures (Deeds et al. 2010). Others have been shown to reduce internal parasite loads (Min and Hart 2003), while others produce aversive post-ingestive feedback and the formation of conditioned food aversions (Distel and Provenza 1991). Unfortunately, variations in the chemical structure and total concentration of tannins are not known for shin oak, blueberry pomace, and grape pomace but may have impacted the differences noted between Trials 1 and 2.

Other considerations for this study may be that animals in were unintentionally exposed to a greater health risk over the course of Trial 1 by the presence of coccidiosis. While bloodwork was collected 7 days after administration of vaccine in Trial 2, it is unknown how the Enterotoxemia Type C and D affects the acquired immune response of goats to the vaccine. A rise in globulins in Trial 1 may indicate the body's activation of an immune response. All antibody molecules are categorized as globulins and typically referred to as immunoglobulins (Tizard 1987). Antibodies are a critical defense mechanism against when battling diseases. They are capable of binding to targeted antigens and labeling them for destruction or removal from the body (Tizard 1987).

Condensed tannins can also affect the nutritive value of a forage due to their ability to bind with proteins. Min et al. (2003) discussed that this binding property can benefit the ruminant animal by allowing forage proteins to escape rumen degradation, which could

increase the absorption of essential amino acids in the small intestine without sacrificing the synthesis of microbial proteins. Enhanced rates of amino acid absorption by the small intestine should increase serum total protein content and possibly globulins. Future research should be attributed toward estimating the ruminal and total tract digestibility of grape pomace compared to other tannin containing sources.

IMPLICATIONS

The goal of this study was to show a positive correlation between a diverse array of chemically rich forages and the health of growing goats. Although a link between CT content and well-being of animals was not facilitated through the pomace rations in Trial 2 of this study, animals on rangelands are exposed to an array of secondary compounds, including tannins, which influence dietary selection and acceptance. The ability of these animals to select from a variety of phytochemicals, such as the CTs in shin oak, may allow animals to make nutritionally wise choices that lead to an enhanced immune response.

LITERATURE CITED

- Brenes, A., Viveros, A., Goñí, I., Centeno, C., Sayago-Ayerdy, S. G., Arija, I., & Saura-Calixto, F. 2008. Effect of grape pomace concentrate and vitamin E on digestibility of polyphenols and antioxidant activity in chickens. *Poultry science*, 87(2), 307-316.
- Cheeke, P. R. 1988. *Natural Toxicants in Feeds and Poisonous Plants*. 2nd Edition.
- Costa, T.D.S.A., Vieira, R.F., Bizzo, H.R., Silveira, D. and Gimenes, M.A. 2012. Secondary metabolites. Embrapa Agroindústria de Alimentos-Capítulo em livro científico (ALICE).
- Deeds, B. E., Scott, C. B., and Brantley, R. 2010. Feeding shinoak to meat goats improves four-wing saltbush and total intake. *Texas Journal of Agriculture and Natural Resources*, 23, 1-11.
- Distel, R. A., & Provenza, F. D. 1991. Experience early in life affects voluntary intake of blackbrush by goats. *Journal of Chemical Ecology*, 17(2), 431-450.
- Dixon, R. 2001. Natural products and plant disease resistance. *Nature* 411, 843–847.
doi:10.1038/35081178
- Dunson, W.T., C.B. Scott, E.S. Campbell, C.A. Taylor, Jr., M.A. Carr, and T.R. Callaway. 2007. Rumen function and the ability of goats to consume redberry juniper (*Juniperus pinchotii*). In: Panter, K.E., T.L. Wierenga, and J.A. Pfister. *Poisonous Plants: Global Research and Solutions*. CABI Publishing, Wallingford, Oxon, UK.
- Freeland, W.J., & Janzen, D.H. 1974. Strategies in herbivory by mammals: the role of plant secondary compounds. *American Naturalist*. 108, 269-289.
- George, C.H., C.B. Scott, T.R. Whitney, C.J. Owens, B.J. May, and R. Brantely. 2010.

- Supplements containing escape protein improve redberry juniper consumption by goats. *Rangeland Ecology and Management*. 63, 655-661.
- Kabera, Justin. 2014. Plant Secondary Metabolites: Biosynthesis, Classification, Function and Pharmacological Classification, Function and Pharmacological Properties. *Journal of Pharmacy and Pharmacology* 2, 377-392.
- Launchbaugh, K.L., C.A. Taylor, E. Straka, and R.K. Pritz. 1997. Juniper as forage: An unlikely candidate? In: 1997 Juniper Symposium. Texas Agr. Exp. Station. Tech Rep. 97-1.
- Lisonbee, L. D., J. J. Villalba, F. D. Provenza, and J. O. Hall. 2009. Tannins and self-medication: Implications for sustainable parasite control in herbivores. *Behavioural Processes* 82,184–189. doi: 10.1016/j.beproc.2009.06.009
- Lozano, G.A. 1998. Parasitic Stress and Self-Medication in Wild Animals. In: Moler, A.P., Milinski, M., Slater, P.J.B. *Advances in the Study of Behavior*, 27. Elsevier Science, London, UK. pp. 291-317.
- McMahon, L. R., T. A. McAllister, B. P. Berg, W. Majak, S. N. Acharya, J. D. Popp, B. E. Coulman, Y. Wang, and K.-J. Cheng. 2000. A review of the effects of forage condensed tannins on ruminal fermentation and bloat in grazing cattle. *Canadian Journal of Plant Science* 80,469–485. doi:10.4141/P99-050.
- Min, B.R. and Hart, S.P., 2003. Tannins for suppression of internal parasites. *Journal of Animal Science*. 81, E102-E109.
- Min, B.R., T.N. Barry, G.T. Attwood, and W.C. McNabb. 2003. The effect of condensed tannins on the nutrition and health of ruminants fed fresh temperate forages: a review.

- Animal feed science and technology. 106, 3-19.
- NRC (National Research Council). 2007. Nutrient requirements of small ruminants: sheep, goats, cervids, and New World camelids. Washington DC, USA: National Research Council. 362 p.
- Provenza, F. D., M. Meuret, and P. Gregorini. 2015. Our landscapes, our livestock, ourselves: Restoring broken linkages among plants, herbivores, and humans with diets that nourish and satiate. *Appetite* 95, 500–519. doi:10.1016/j.appet.2015.08.004.
- Provenza, F.D., Villalba, J.J. 2010. The Role of Natural Plant Products in Modulating the Immune System: An Adaptable Approach for Combating Disease in Grazing Animals. *Small Ruminant Research* 89:131-139. doi:10.1016/.smallrumres.2009.12.035.
- Rosenthal, G.A., Berenbaum, M.R. 1992. *Herbivores: Their Interactions with Secondary Plant Metabolites*, Second Ed. Academic Press, New York, p. 493
- Ross, K. A., D. Ehret, D. Godfrey, L. Fukumoto, and M. Diarra. 2017. Characterization of Pilot Scale Processed Canadian Organic Cranberry (*Vaccinium macrocarpon*) and Blueberry (*Vaccinium angustifolium*) Juice Pressing Residues and Phenolic-Enriched Extractives. *International Journal of Fruit Science* 17:202–232. doi: 10.1080/15538362.2017.1285264.
- Smith, G.S. 1992. Toxification and detoxification of plant compounds by ruminants: An overview. *Journal of. Range Manage.* 45,25–30.
- Tizard, I. 1987. *Veterinary Immunology an introduction*. Ed. 3.

- Villalba, J. J., F. D. Provenza, and G. Han. 2004. Experience influences diet mixing by herbivores: implications for plant biochemical diversity. *Oikos* 107,100–109. doi: 10.1111/j.0030-1299.2004.12983.x.
- Villalba J.J., Provenza F.D. 2009. Learning and dietary choice in herbivores. *Rangeland Ecology and Management* 62,399–406.
- Waghorn, G. C., G. B. Douglas, J. H. Niezen, W. C. McNabb, and A. G. Foote. 1998. Forages with condensed tannins-their management and nutritive value for ruminants. *Proceedings of the New Zealand Grassland Association*. 60, 89-98.

APPENDIX



ANGELO STATE UNIVERSITY

College of Graduate Studies & Research

Institutional Animal Care & Use Committee

April 15, 2020

Cody Scott, Professor
Agriculture
Angelo State University
ASU Station #10888
San Angelo, TX 76909

Your proposed project titled, “Effects of Phytochemical Diversity on the health of Growing Goats” was reviewed by Angelo State University’s Institutional Animal Care and Use Committee (IACUC) in accordance with the regulations set forth in the Animal Welfare Act and P.L. 99-158.

This protocol was approved for three years, effective May 9, 2021 and it expires three years from this date; however, an annual review and progress report form (www.angelo.edu/content/files/22583-iacuc-annual-review-progressreport) for this project is due on August 15 of each year. If the study will continue beyond three years, you must submit a request for continuation before the current protocol expires.

The protocol number for your approved project is 2020-105. Please include this number in the subject line of in all future communications with the IACUC regarding the protocol.

Sincerely,

A handwritten signature in blue ink that reads 'Chase Runyan'.

Chase Runyan, Ph.D.
Co-Chair, Institutional Animal Care and Use Committee

VITA

Rebecca Deann Burson is the daughter of Marc and Carol Burson of Tuscola, Texas. She received her Bachelor of Science in Animal Science from Texas Tech University in 2013. After graduation, Deann gained multiple years of experience working in the industry including a position at the Texas A&M AgriLife's Grazing Animal Nutrition Laboratory in Temple, Texas. In 2019, Deann made the decision to return to school at Angelo State University for a Master of Science in Animal Science. She accepted a graduate research assistantship at Angelo State University, where she worked for the Management, Instruction, and Research (MIR) Center. During her master's program, Deann was also employed by the Texas A&M AgriLife Center in San Angelo, Texas under the direction of the Range Extension Specialist, Dr. Morgan Treadwell. Deann was admitted into the Doctor of Philosophy program at Texas A&M University and was selected as a recipient of the Wasko Graduate Fellowship by the Department of Rangeland, Wildlife & Fisheries Management. She will begin her fellowship in the Fall of 2021. Upon completion of the program, Deann seeks to pursue a career in rangeland research or extension. Her main research interests include ruminant diet selection, brush management, and prescribed fire.